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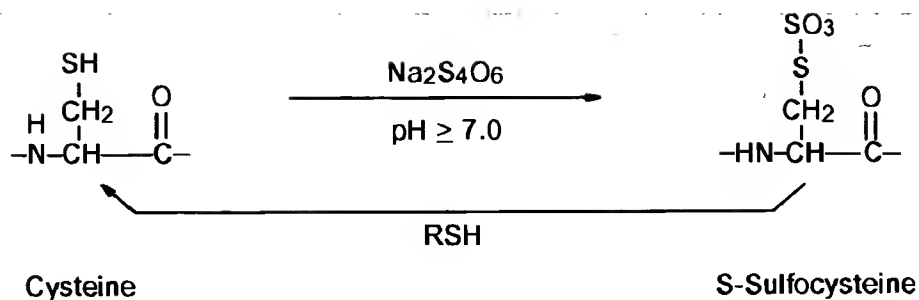
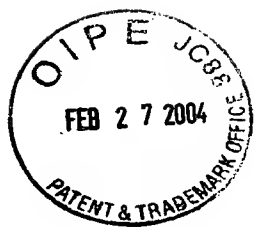
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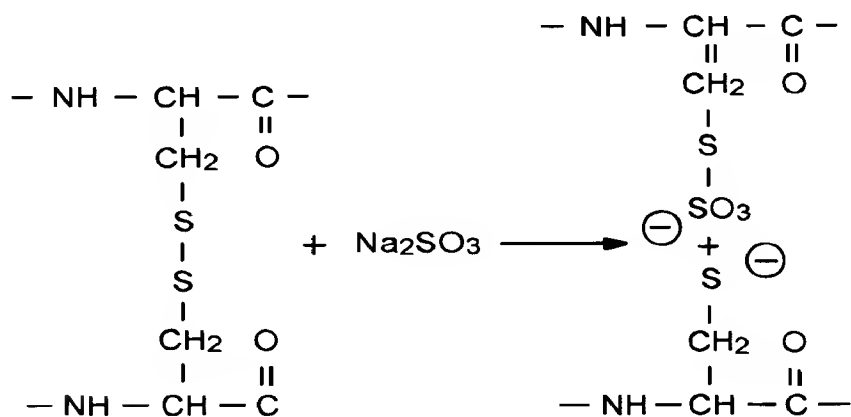
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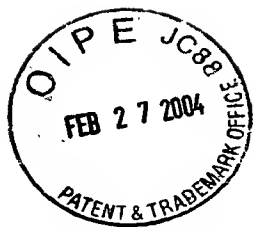
Conversion of cysteine to S-sulfocysteine by reaction with sodium tetrathionate and reversal by exogenous thiols

FIG. 1A



The Cleavage of disulfide bonds by sodium sulfite to form the S-sulfo derivative

FIG. 1B



Preparation and Washing of TnI-containing Inclusion Bodies

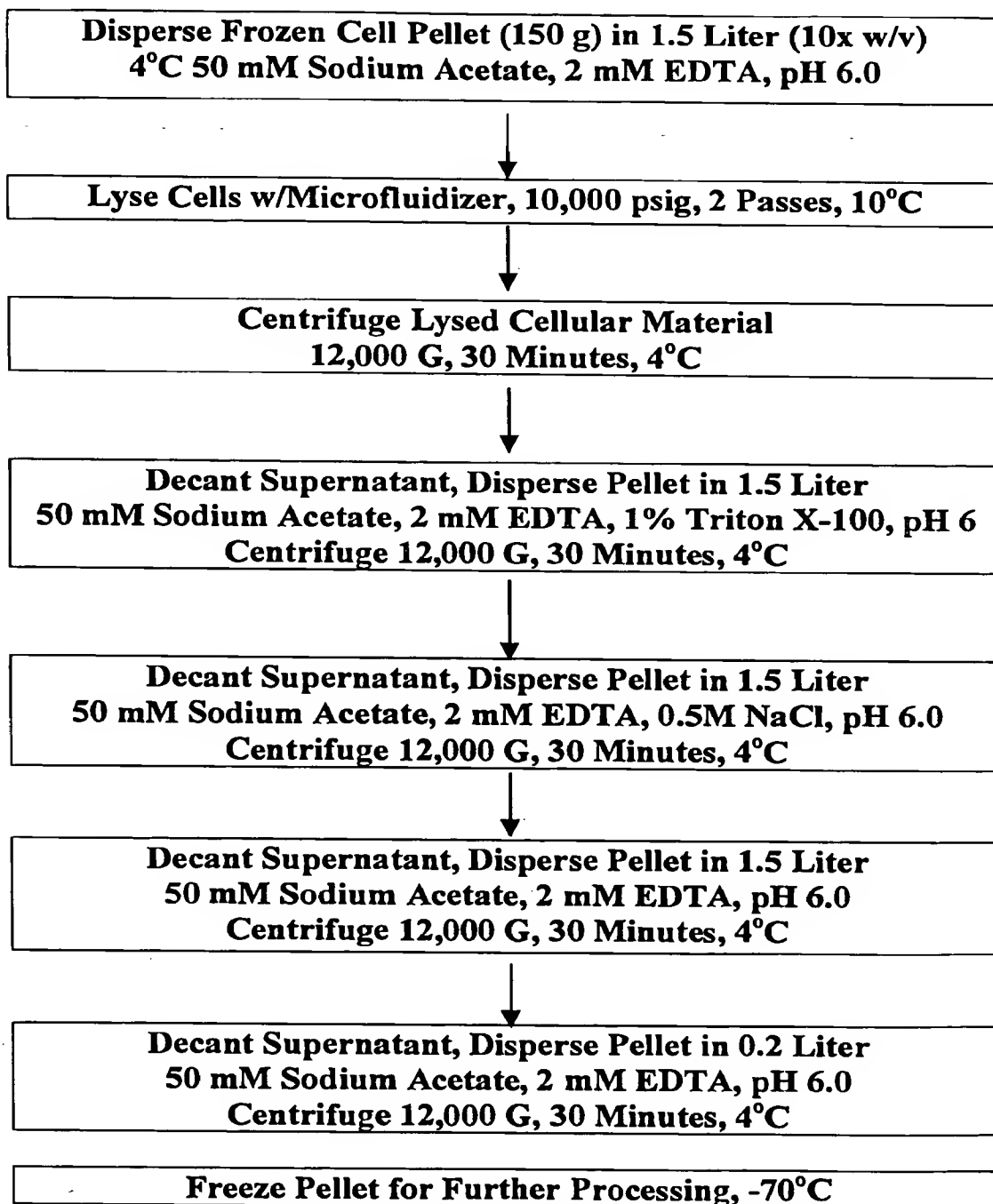
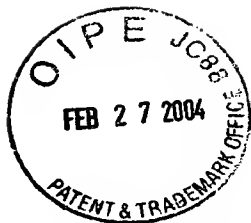


FIG. 2



Summary of rTroponin-I Preparation – 3L5 (050900-051200)

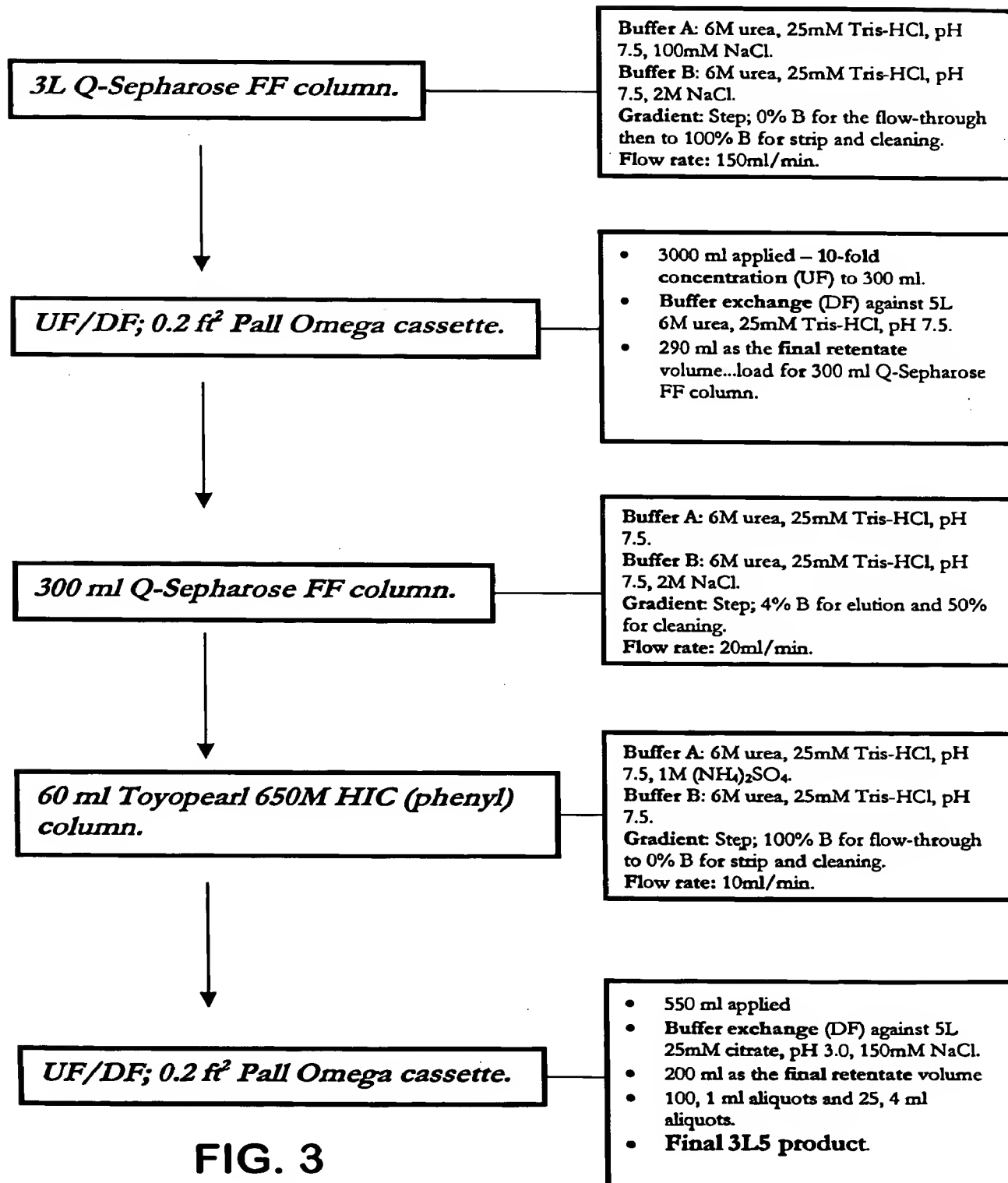
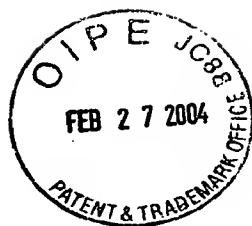


FIG. 3



3L5: 3L Q-Sepharose FF

3L5: 3L Q-Sepharose FF (Q1)

Buffer A: 6M urea, 25mM Tris-HCl, pH 7.5, 100mM NaCl.

Buffer B: 6M urea, 25mM Tris-HCl, pH 7.5, 2M NaCl
Gradient: Step; 0% B for the flow-through and 100% B for the strip.

Flow rate: 150ml/min.

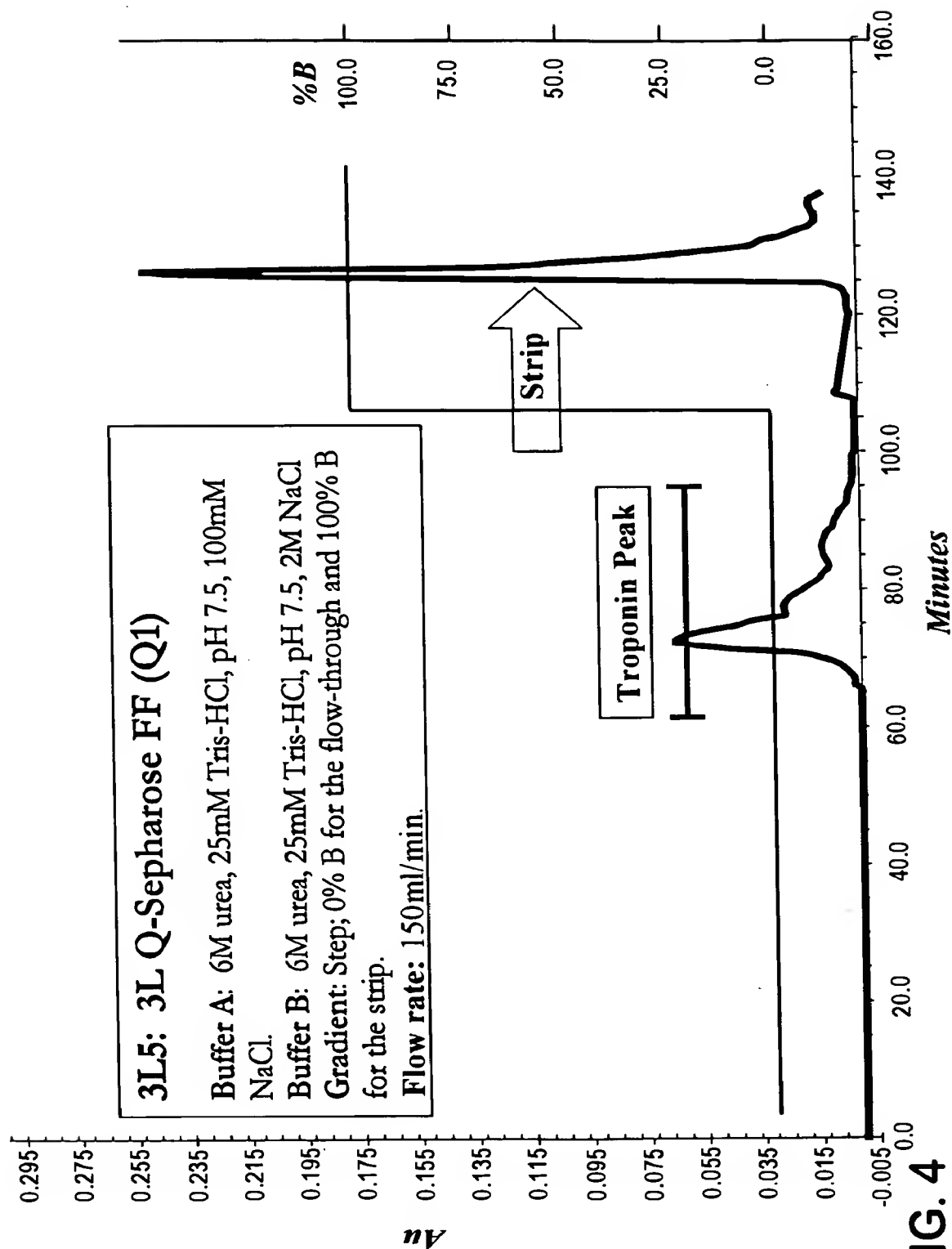


FIG. 4

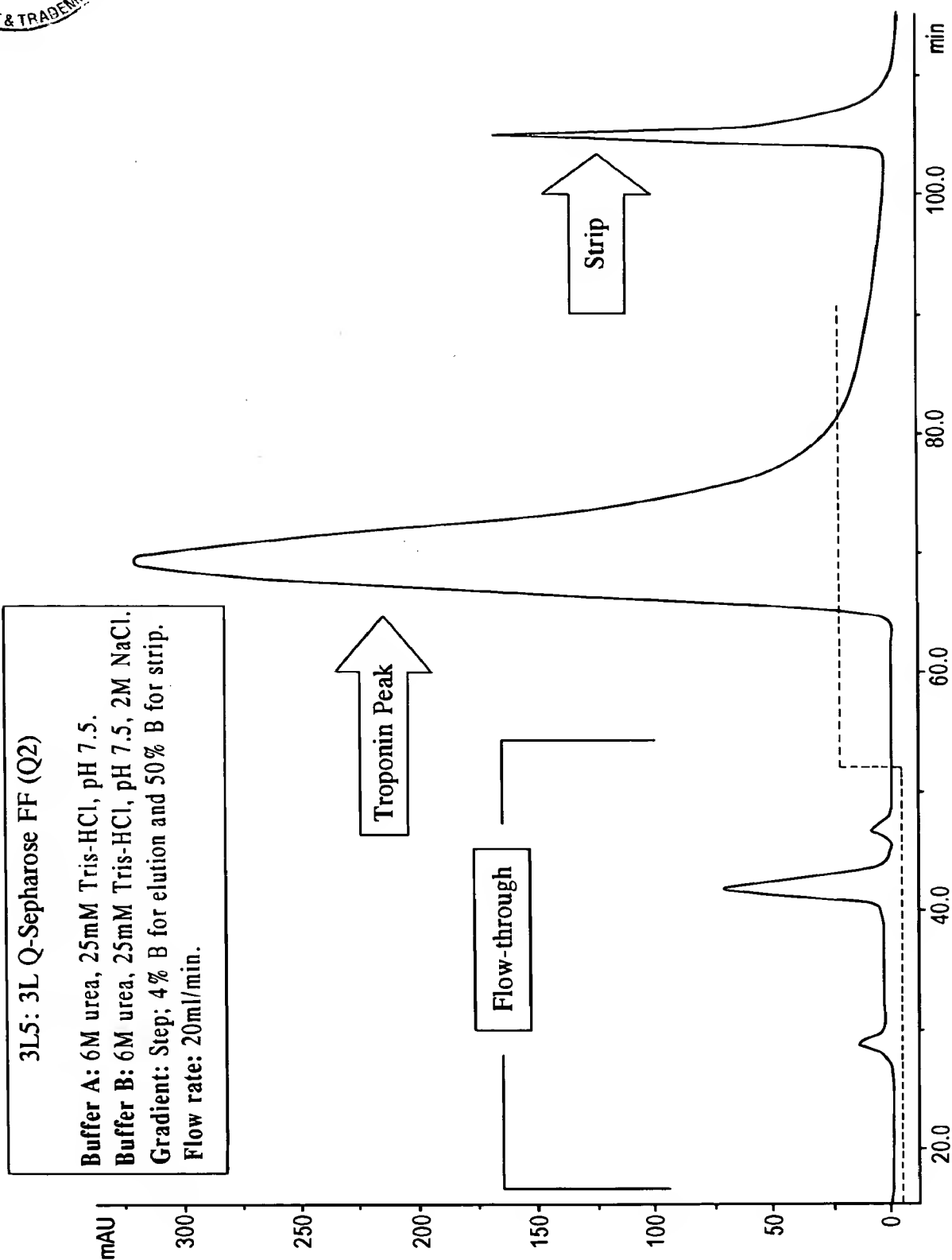
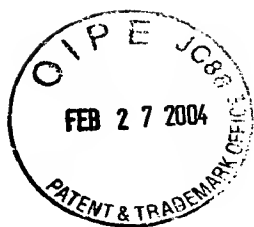
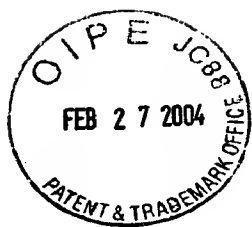
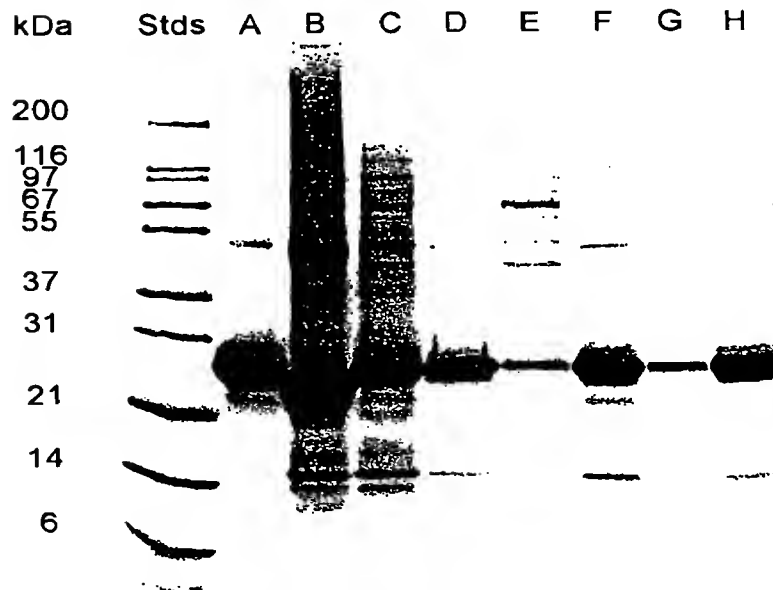


FIG. 5



SDS-PAGE Analysis Troponin Lot 3L5
Anion Exchange Steps #1 and #2
16% Tris-glycine Gel, Non-reducing, 5/15/00



- A. Sulfitolyzed Troponin Lot 3L4 Standard
- B. Solublized Inclusion Bodies
- C. Sulfitolyzed Inclusion Bodies (AEX #1 Load)
- D. Anion Exchange #1 Flowthrough
- E. Anion Exchange #1 Salt Eluate
- F. Anion Exchange #2 Load
- G. Anion Exchange #2 Flowthrough
- H. Anion Exchange #2 100 mM NaCl Eluate

FIG. 6

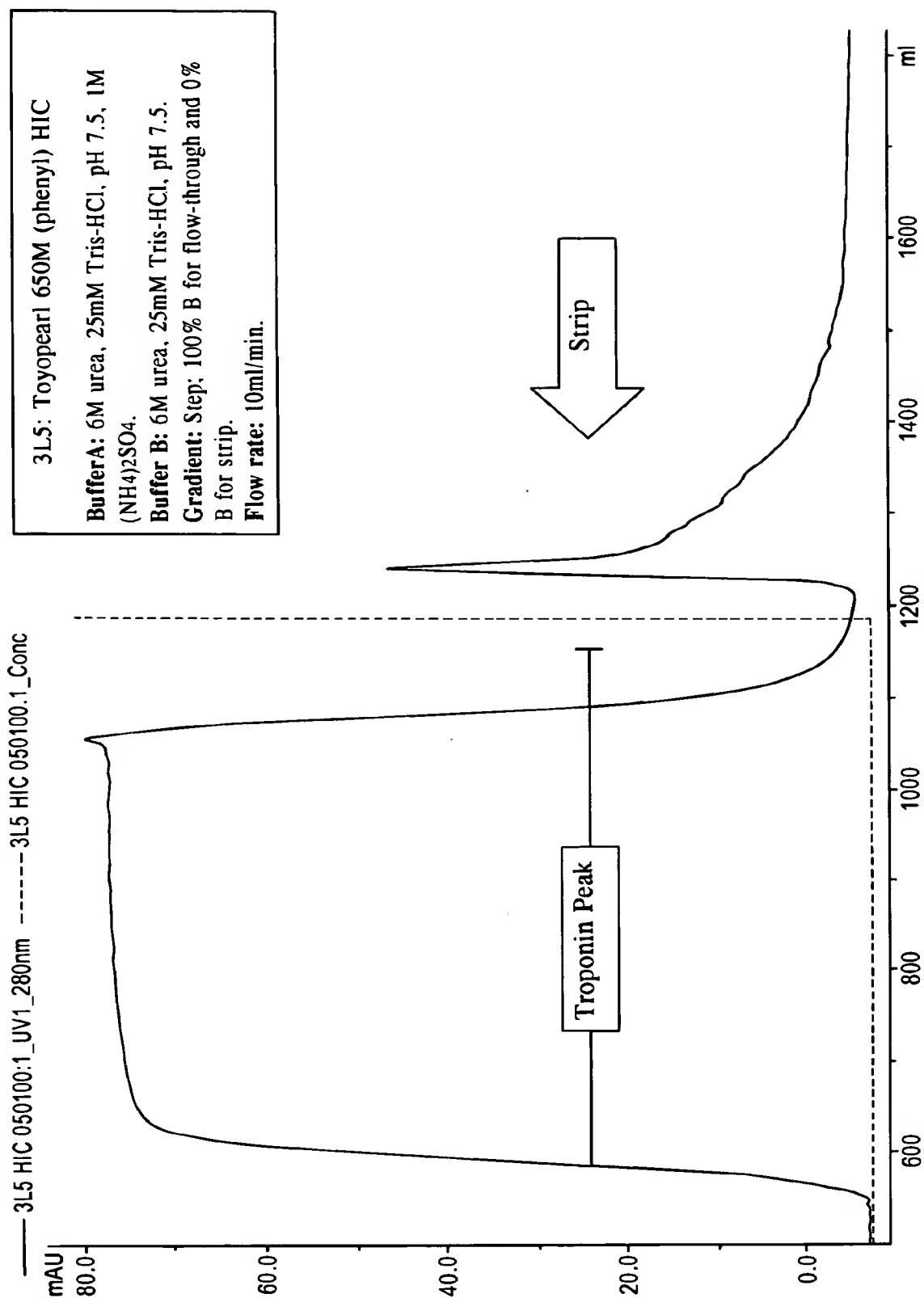


FIG. 7

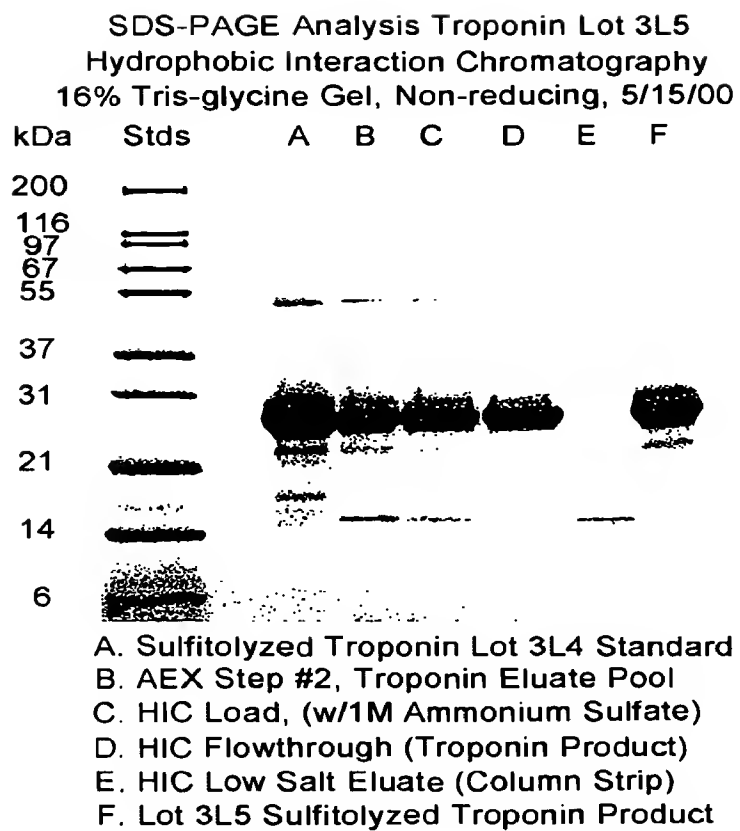
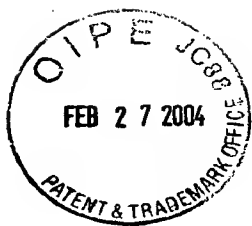
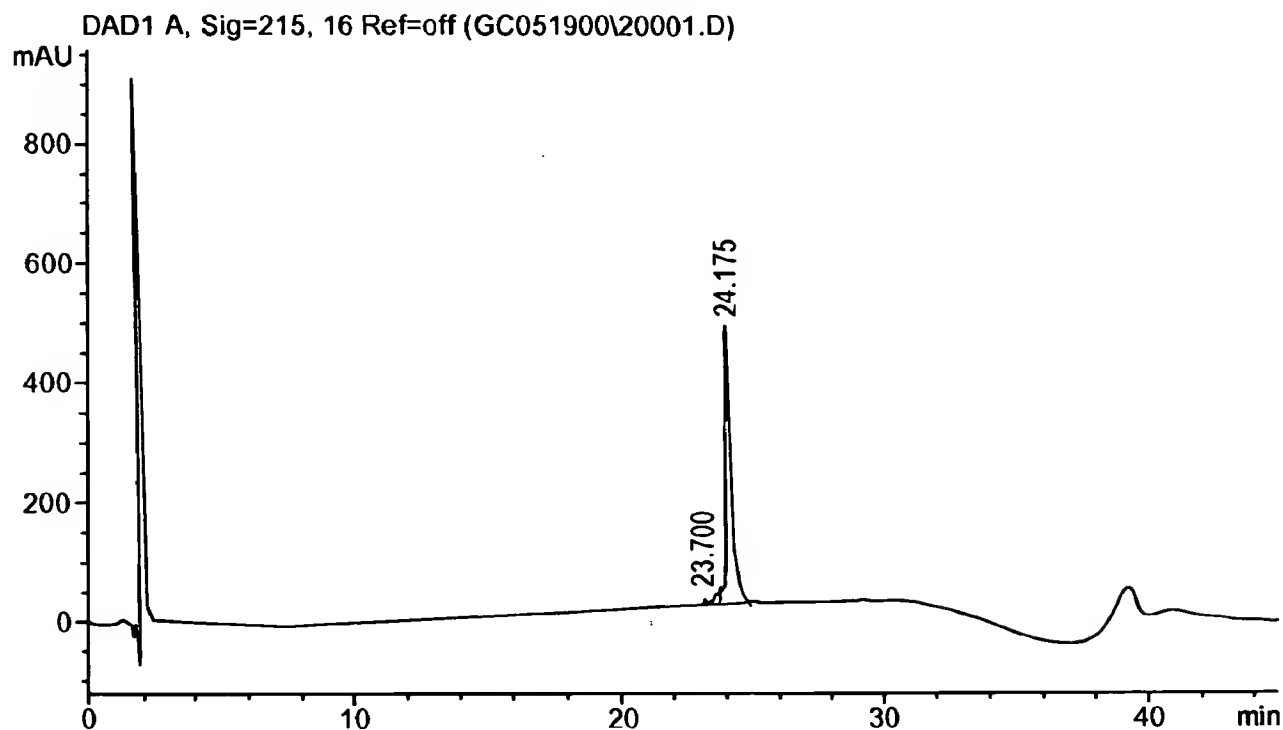


FIG. 8



Acq. Method : TROP3Q.M
Analysis Method : C:\HPCHEM\1\METHODS\TROP3Q.M
Last Changed : 03:04:24 pm
(modified after loading)
Quantitation of rTnI on Zorbax C3



Customized Report: gc

Sorted By RT

Calib. Data Modified : 3:53 am

Multiplier : 1.000000

Dilution : 1.000000

Uncalibrated Peaks : not reported

Peak #	RT [min]	Width [min]	Amount micrograms	Area [mAU*s]	Area %	Name
1	23.70	0.23	0.00	343.975	6	Troponin
2	24.17	0.18	2.08	5893.704	94	TROPONIN 3L5

FIG. 9



troponin I

LysC mapping

#	RC	Mass	Sequence
1	-2.1	707.28	- MGDEEK (1,6)
2	5.2	1618.96	- RNRAITARRQHLK (7,19)
3	30.9	1431.76	- SVM LQIAATELEK (20,32)
4	-4.8	1132.55	- EESRREAEEK (33,41)
5	48.0	2824.26	- QNYLAEHCPPLHIPGSMSEVQELCK (42,66)
6	-1.8	595.34	- QLHAK (67,71)
7	5.1	903.42	- IDAAEEEEK (72,79)
8	10.9	1166.58	- YDMEVRVQK (80,88)
9	-9.4	334.19	- TSK (89,91)
10	5.6	1005.44	- ELEDMNQK (92,99)
11	13.9	847.49	- LFDLRGK (100,106)
12	-1.7	293.17	- FK (107,108)
13	28.0	1896.07	- RPPLRRVRMSADAMLK (109,124)
14	8.0	587.36	- ALLGSK (125,130)
15	-11.9	283.16	- HK (131,132)
16	20.5	1161.60	- VCMDLRANLK (133,142)
17	-4.8	373.23	- QVK (143,145)
18	-9.8	146.11	- K (146,146)
19	-6.8	620.27	- EDTEK (147,151)
20	15.7	1543.79	- ERDLRDVG DWRK (152,163)
21	-0.8	631.32	- NIEEK (164,168)
22	-4.4	763.36	- SGMEGRK (169,175)
23	-9.8	146.11	- K (176,176)
24	7.7	728.27	- MFESES (177,182)

Current Chromatogram(s)

DAD1 C, Sig=218, 8 Ref=360, 100 (GC051500\3L5LYS2.D)

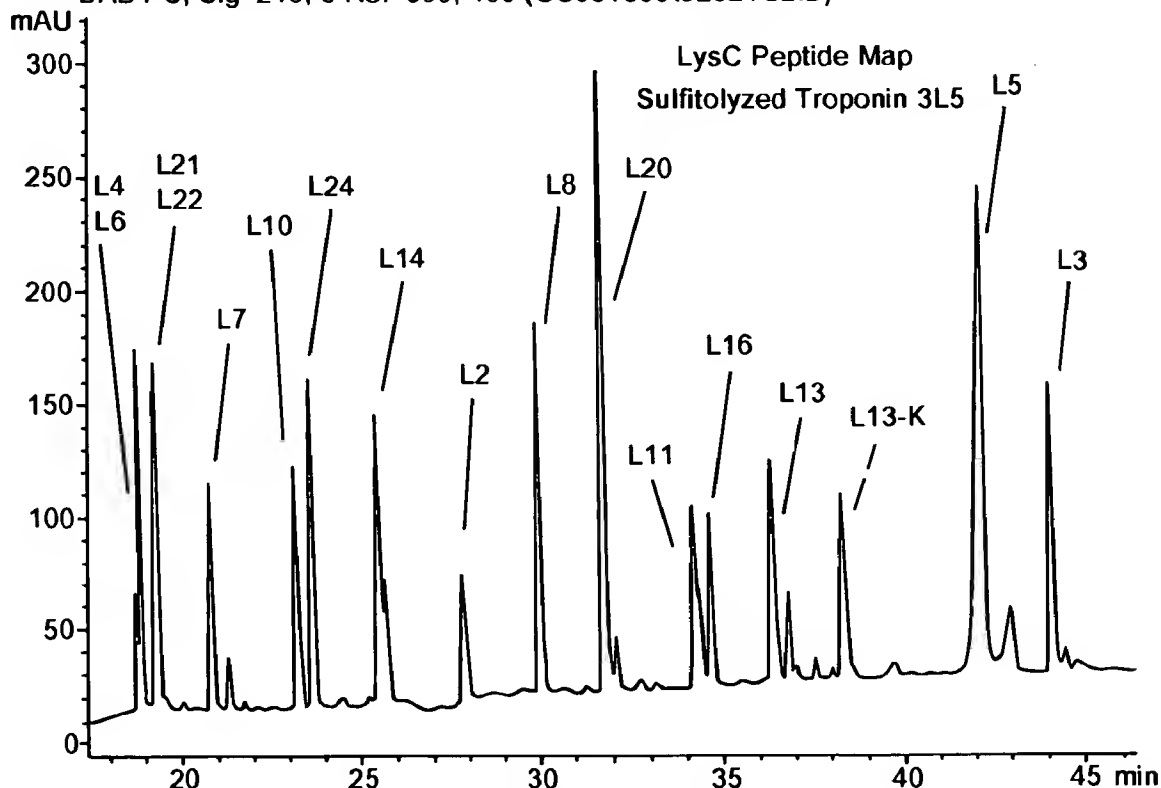


FIG. 10



SDS-PAGE Analysis Sulfitolyzed Troponin Reduction w/DTT

45 Minutes, Ambient Temperature, 1 mg.ml Tnl

6M Urea, 25 mM Tris, 0.15M NaCl pH 7.5

01/08/2000, 16% Tris-glycine Gel

1 2 3 4 5 6 7 8 9 10

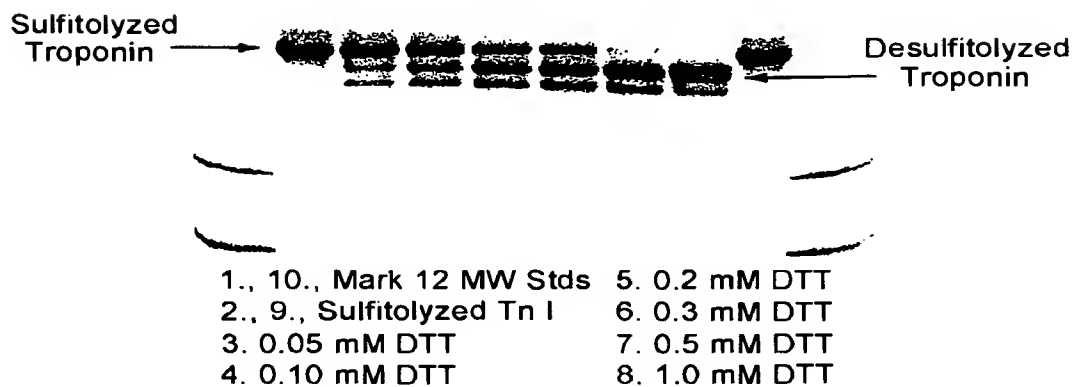


FIG. 11